TITLE: *Veillonella parvula* ENHANCE METABOLIC ACTIVITY OF Streptococcus mutans BIOFILMS AGAINST DIFFERENT ANTIMICROBIALS

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ABSTRACT:

The aim of this study was to evaluate the effect of Veillonella parvula (VP) on antimicrobial sensitivity of Streptococcus mutans (SM) in dual species biofilm using a novel metabolic activity indicator. SM UA159 containing vector pDM15 (expressing green fluorescent protein; GFP) and VP DSM2008 were the strains used in this study. Single SM and dual SM and VP biofilms were grown in a 96-well transparent bottom black microtiter plate and incubated anaerobically at 37°C, with the SDMY medium being refreshed 8 h after inoculation. After 24 h of biofilm growth, treatments with the following antimicrobials were performed: Sodium Fluoride at 1, 1.8, 2.4 and 3.6 mM; Cetylpyridinium chloride at 0.0125, 0.05, 0.2 and 0.8 mM and Chlorhexidine at 0.00125, 0.0025, 0.005 and 0.01%. Also, a placebo/control solution (containing only the medium that antimicrobials were prepared) was tested. SM fluorescence intensities (FI) were evaluated for 3 h after additions at room temperature. All experiments were done in triplicate. Fluorescence reduction (%FR) of all treatments was calculated from the area under the FI curve. ANOVA was used to analyze the effect of the antimicrobials on %FR of biofilms and comparisons between single and dual species biofilms were performed using a paired t test, with p fixed at 5%. Overall FI decreased with increasing concentrations of all antimicrobials tested in both single and dual biofilms, however in dual species biofilms %FR decreased to a higher extent than in single species biofilms, indicating higher metabolic activity during all antimicrobial titrations in dual species biofilms. Thus, metabolic activity of SM in single or dual species biofilms is clearly affected by the antimicrobials used. Furthermore, Veillonella parvula increases antimicrobial resistance of Streptococcus mutans as shown by metabolic activity estimated by constitutive expression of GFP.

Keywords: Veillonella parvula, Streptococcus mutans, biofilms, antimicrobials.

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